

What is claimed is:

1. A method for determining the identity of a nucleotide present at a predetermined site in a DNA whose sequence immediately 3' of such predetermined site is known which comprises:
 - (a) treating the DNA with an oligonucleotide primer whose sequence is complementary to such known sequence so that the oligonucleotide primer hybridizes to the DNA and forms a complex in which the 3' end of the oligonucleotide primer is located immediately adjacent to the predetermined site in the DNA;
 - (b) simultaneously contacting the complex from step (a) with four different labeled dideoxynucleotides, in the presence of a polymerase under conditions permitting a labeled dideoxynucleotide to be added to the 3' end of the primer so as to generate a labeled single base extended primer, wherein each of the four different labeled dideoxynucleotides (i) is complementary to one of the four nucleotides present in the DNA and (ii) has a molecular weight which can be distinguished from the molecular weight of the other three labeled dideoxynucleotides using mass spectrometry; and
 - (c) determining the difference in molecular weight between the labeled single base extended primer and the oligonucleotide primer so as to identify the dideoxynucleotide incorporated into the single base extended primer and thereby determine the identity of the

nucleotide present at the predetermined site in the DNA.

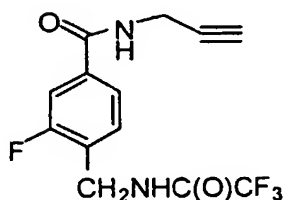
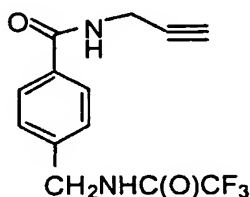
- 5 2. The method of claim 1, wherein each of the four labeled dideoxynucleotides comprises a chemical moiety attached to the dideoxynucleotide by a different linker which has a molecular weight different from that of each other linker.
- 10 3. The method of claim 1 which further comprises after step (b) the steps of:
 - 15 (i) contacting the labeled single base extended primer with a surface coated with a compound that specifically interacts with a chemical moiety attached to the dideoxynucleotide by a linker so as to thereby capture the extended primer on the surface; and
 - (ii) treating the labeled single base extended primer so as to release it from the surface.
- 20 4. The method of claim 3 which further comprises after step (i) the step of treating the surface to remove primers that have not been extended by a labeled dideoxynucleotide.
- 25 5. The method of claim 1, wherein step (c) comprises determining the difference in mass between the labeled single base extended primer and an internal mass calibration standard added to the extended primer.
- 30 6. The method of claim 3, wherein the interaction between the chemical moiety attached to the

dideoxynucleotide by the linker and the compound on the surface comprises a biotin-streptavidin interaction, a phenylboronic acid-salicylhydroxamic acid interaction, or an antigen-antibody interaction.

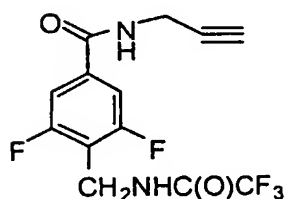
- 5 7. The method of claim 3, wherein the step of releasing the labeled single base extended primer from the surface comprises disrupting the interaction between the chemical moiety attached to the dideoxynucleotide by the linker and the compound on the surface.
- 10 8. The method of claim 7, wherein the interaction is disrupted by a means selected from the group consisting of one or more of a physical means, a chemical means, a physical chemical means, heat, and light.
- 15 9. The method of claim 2, wherein the linker is attached to the dideoxynucleotide at the 5-position of cytosine or thymine or at the 7-position of adenine or guanine.
- 20 10. The method of claim 3, wherein the step of releasing the labeled single base extended primer from the surface comprises cleaving the linker between the chemical moiety and the dideoxynucleotide.
- 25 11. The method of claim 10, where the linker is cleaved by a means selected from the group consisting of one or more of a physical means, a chemical means, a physical chemical means, heat, and light.
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12. The method of claim 11, wherein the linker is cleaved by light.
- 5 13. The method of claim 2, wherein the linker comprises a derivative of 4-aminomethyl benzoic acid, a 2-nitrobenzyl group, or a derivative of a 2-nitrobenzyl group.
- 10 14. The method of claim 13, wherein the linker comprises one or more fluorine atoms.

15. The method of claim 14, wherein the linker is selected from the group consisting of:



and



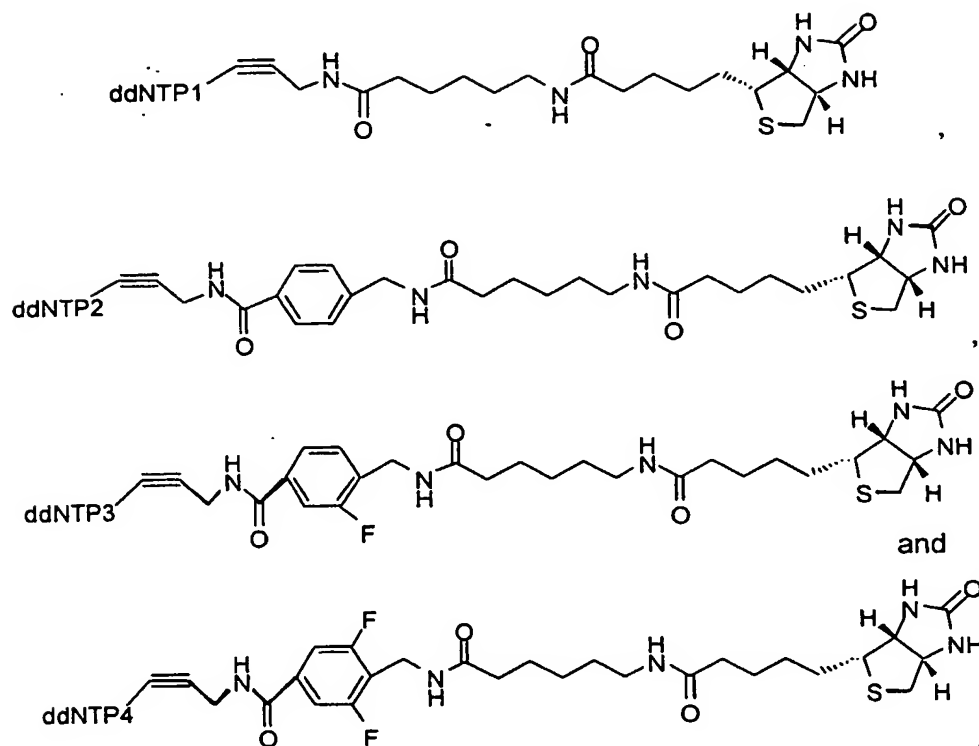
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16. The method of claim 3, wherein the chemical moiety comprises biotin, the labeled dideoxynucleotide is a biotinylated dideoxynucleotide, the labeled single base extended primer is a biotinylated single base extended primer, and the surface is a streptavidin-coated solid surface.

17. The method of claim 16, wherein the biotinylated dideoxynucleotide is selected from the group consisting of ddATP-11-biotin, ddCTP-11-biotin, ddGTP-11-biotin, and ddTTP-16-biotin.

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18. The method of claim 16, wherein the biotinylated dideoxynucleotide is selected from the group consisting of:

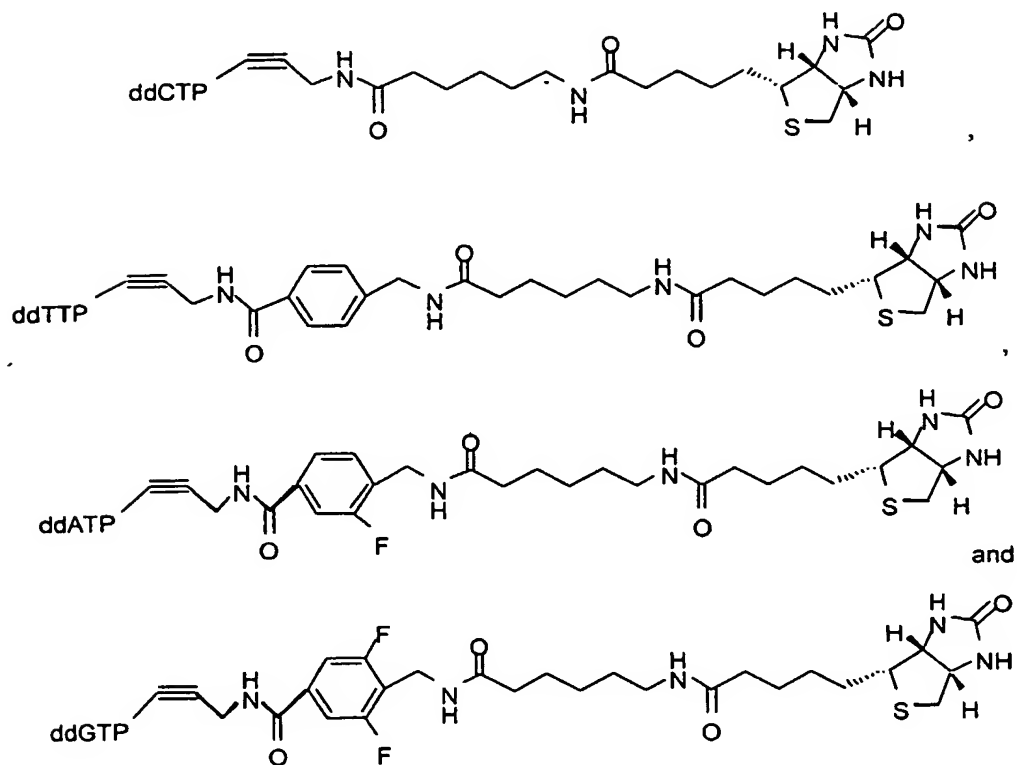


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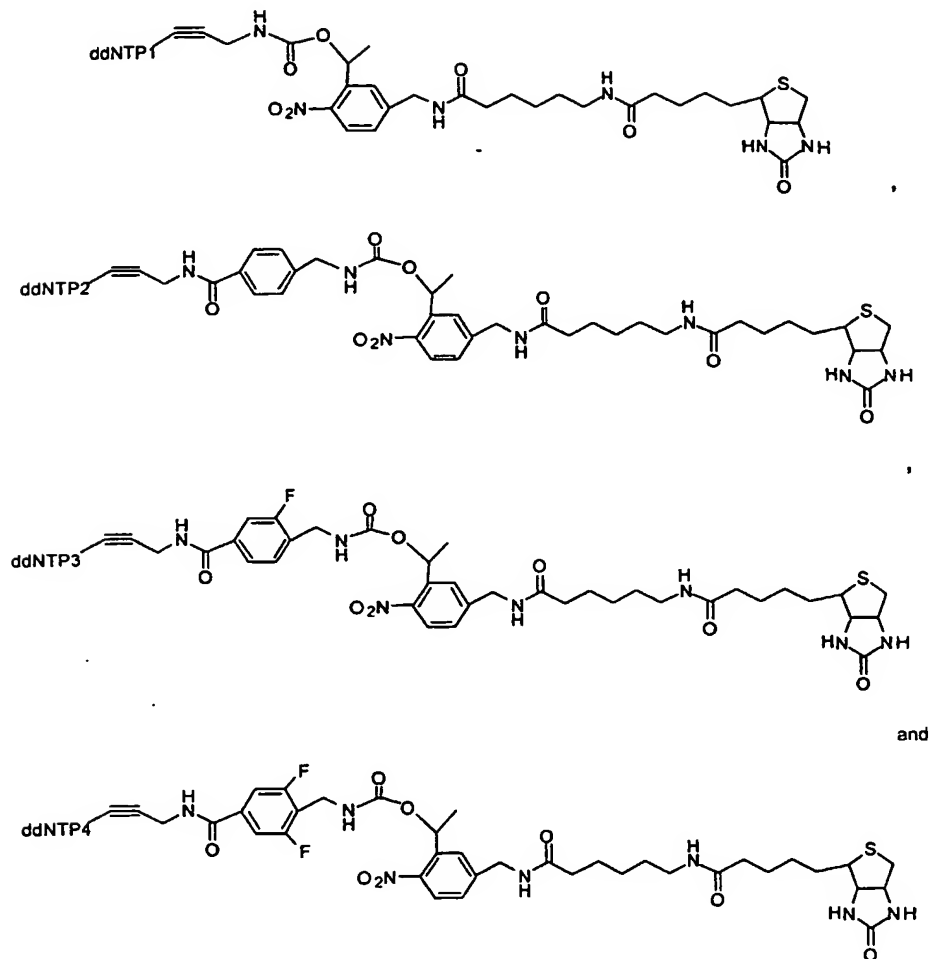
wherein ddNTP1, ddNTP2, ddNTP3, and ddNTP4 represent four different dideoxynucleotides.

19. The method of claim 18, wherein the biotinylated dideoxynucleotide is selected from the group consisting of:

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20. The method of claim 16, wherein the biotinylated dideoxynucleotide is selected from the group consisting of:

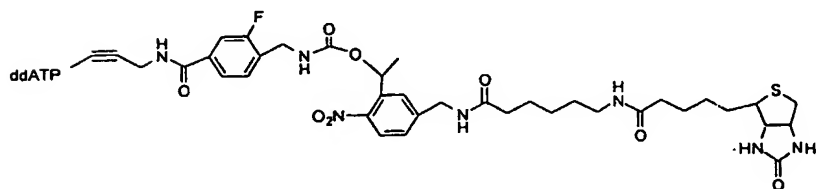
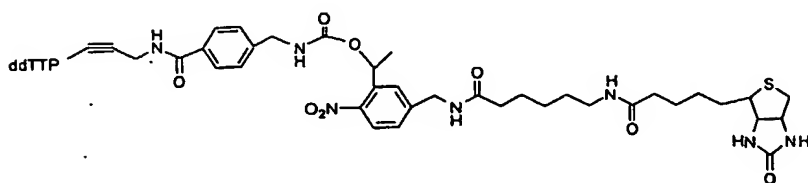
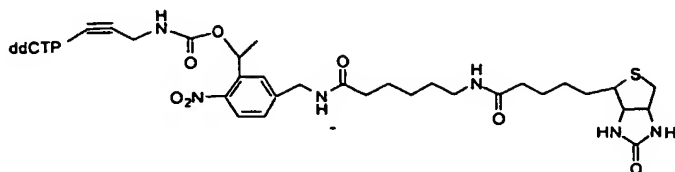


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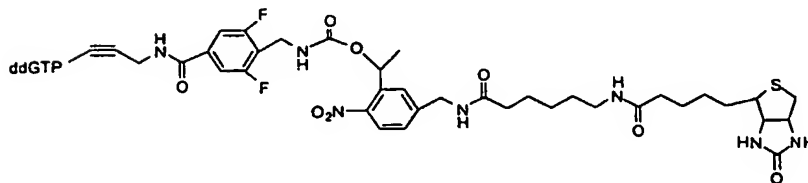
wherein ddNTP1, ddNTP2, ddNTP3, and ddNTP4 represent four different dideoxynucleotides.

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21. The method of claim 20, wherein the biotinylated dideoxynucleotide is selected from the group consisting of:



and



22. The method of claim 16, wherein the streptavidin-coated solid surface is a streptavidin-coated magnetic bead or a streptavidin-coated silica glass.
- 5 23. The method of claim 1, wherein steps (a) and (b) are performed in a single container or in a plurality of connected containers.
- 10 24. A method for determining the identity of nucleotides present at a plurality of predetermined sites, which comprises carrying out the method of claim 3 using a plurality of different primers each having a molecular weight different from that of each other primer, wherein a different primer hybridizes
15 adjacent to a different predetermined site.
- 20 25. The method of claim 24, wherein different linkers each having a molecular weight different from that of each other linker are attached to the different dideoxynucleotides to increase mass separation between different labeled single base extended primers and thereby increase mass spectrometry resolution.